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Synthesis and Antimicrobial Activity of Nitrobenzaldehyde Schiff base and Complexes

O. Akeredolu[1], F. O. Adebusuyi[1], B. C. Adebayo-Tayo[2] and T. E. Olalekan[1*]

[1]Department of Chemistry, Faculty of Science, University of Ibadan, 200285 Oyo State, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Ibadan, 200285 Oyo State, Nigeria.
*Correspondence Author E-mail: topeolalekan11@yahoo.com

ABSTRACT

The Schiff base (NL) was synthesized by the reaction between ethanolamine and 3-nitrobenzaldehyde. The metal complexes with Mn(II), Co(II), Ni(II), and Cu(II) were obtained in an alcoholic KOH medium. The diagnostic H-C=N proton shift at 8.48 δ ppm confirmed the formation of the Schiff base ligand. The stoichiometry of the metal complexes was determined through the percentage metal content by EDTA titration. The infrared spectra suggested the chelation of the ligand through the ethanolamine oxygen as a monodentate ligand. The O-H band of the hydroxide ion was observed between 3650 and 3450 cm^{-1}. The shift in $n \rightarrow \pi^*$ transition from 327 nm in the ligand spectrum to 337-347 nm in the complexes was ascribed to delocalisation of lone electrons to the aromatic ring upon coordination, while the metal ions were assigned to the octahedral geometry as shown by their visible bands. The in vitro antimicrobial potency of the compounds was investigated against ten microbial strains using the agar well diffusion method. The compounds were generally active against the bacterial and fungal cells, demonstrating a broad-spectrum while the Cu(II) complex has the largest inhibition zone against Staphylococcus aureus.

Keywords: Schiff base; Metal complexes; Infrared spectra; Antimicrobial activity

INTRODUCTION

Schiff base ligands are typically formed by reaction of aldehydes or ketones with primary amines. The study of these compounds continues to attract research interest as they are readily prepared, giving vast array of possible structures. Schiff bases have been applied in medicinal and pharmacological fields for antitubercular,[1] anticancer[2] and antimicrobial studies,[3] explored as catalysts substrates,[4] in green chemistry[5, 6] and in synthetic reaction studies.[7, 8]

The search for small molecules with bioactivity has been progressive because of their advantage in gaining easier access to the cell walls. Small-molecule drugs are known to serve as effectors and alter the functions of a target and bind specific biological macromolecules. Our study is aimed at attempting to synthesize low molecular weight compounds and investigate their antimicrobial potency. The synthesis and *in vitro* antimicrobial studies of a nitrobenzaldehyde Schiff base and its Mn(II), Co(II), Ni(II) and Cu(II) complexes are hereby reported in this work.

MATERIALS AND METHODS

Materials and Measurements

The materials and chemicals of analytical grades were purchased from Sigma-Aldrich and used without further purification. The melting points (uncorrected) were determined using Gallenkamp melting point apparatus The IR spectra of the samples were recorded as nujol mulls on a PerkinElmer FT-IR spectrophotometer in the range 4000–350 cm^{-1}. ^1H and ^{13}C NMR spectra were obtained in d_6-DMSO and recorded on Bruker Avance 400 MHz NMR spectrometer; tetramethylsilane was used as the internal standard. The electronic spectra of the compounds were obtained in the UV-Vis range 400-190 nm on Labomed double beam UV-visible spectrophotometer and by the diffuse reflectance method on a Cary-5000 Varian spectrophotometer (40000-5000 cm^{-1}). Percentage metal content was determined after digestion of the metal complexes and EDTA titration.

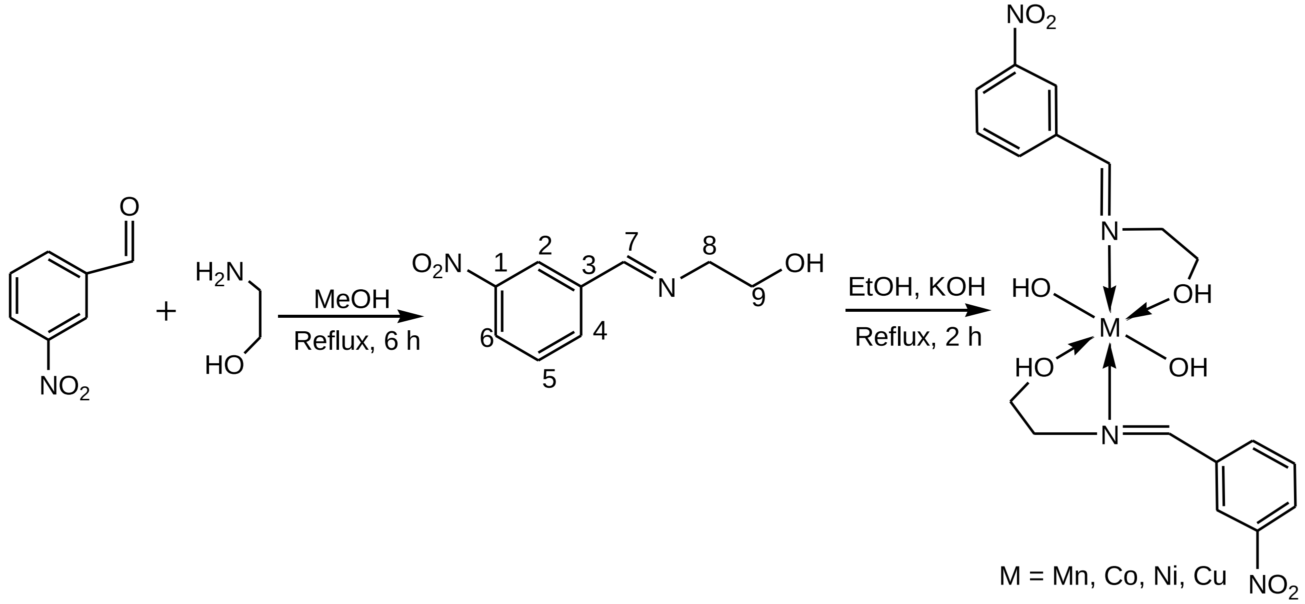
In vitro antimicrobial assay procedure

The synthesized compounds were screened for *in vitro* antibacterial activity by the Agar well diffusion method against six (6) bacteria; *Escherichia coli* ATCC 25922 (*E. c*), *Staphylococcus aureus* ATCC 6538 (*S. a*), *Bacillus subtilis* ATCC 6633 (*B. s*), *Klebsiella pneumonia* ATCC 13883 (*K. p*), *Pseudomonas aeruginosa* ATCC 29853 (*P. a*), *Salmonella typhi* ATCC 33458 (*S. t*), and *in vitro* antifungal activity against four (4) fungi; *Candida albicans* ATCC 10231 (*C. a*), *Aspergillus niger* ATCC 6275 (*A. n*), *Penicillium notatum* ATCC 28089 (*P. n*) and *Rhizopus stolonifer* ATCC 14037 (*R. s*). Gentamicin 10 µg/mL (antibacterial) and tioconazole 70% (antifungal) were prepared as standard solutions, 1000 µg/mL of each compound was used as the initial concentration which was serially diluted in double fold with DMSO to give other concentrations; 500, 250, 125, 62.5, 31.25 µg/mL. DMSO solvent was used as the negative control for the experiment.

Cultures of each organism and preparation of agar media (nutrients broth, nutrient agar and Sabouraud dextrose agar) were carried out according to standard methods. 8 mm-sized wells were made into the media surfaces with a sterile cork borer and the graded concentrations of the compounds and controls were introduced into the wells, these were done in duplicates. The plates were incubated in the incubator at 26–28˚C, the bacterial test plates were observed after 24 h of incubation while the fungal plates were also observed after 48 h.

Synthesis of the ligand (NL)

The synthesis of the ligand was carried out as depicted in Scheme 1, ethanolamine (2.00 mL, 33.10 mmol) was added drop-wise to a stirring solution of 3-nitrobenzaldehyde (5.00 g, 33.10 mmol) in MeOH (10 mL). The mixture was heated to reflux on a magnetic stirrer hot plate for 6 h. The precipitate was collected by filtration, washed and dried and the mass of solid product was 5.93 g (90%). Mp 98-99°C, mol. wt: 194.20. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 8.56 (s, 1 H, H2), 8.48 (s, 1 H, H7), 8.30 (d, 1 H, H6, $J_{6\text{-}5}$ = 8 Hz), 8.18 (d, 1 H, H4, J = 4 Hz), 7.75 (t, 1 H, H5, $J_{5\text{-}6}$ = 8 Hz), 4.68 (s, 1H, -OH), 3.69 (s, 4 H, -CH$_2$–CH$_2$-); ^{13}C NMR (400 MHz, DMSO): δ = 60.99 (C9), 63.56 (C8), 122.27 (C6), 125.36 (C2), 130.81 (C5), 134.64 (C4), 138.21 (C3), 148.61 (C1), 160.47 (C7) ppm.



Scheme 1: Synthesis of the NL Schiff base and metal(II) complexes

Synthesis of metal(II) complexes

The general method for the preparation of the metal complexes was shown in Scheme 1. A mixture of NL (0.50 g, 2.57 mmol) and potassium hydroxide (0.14 g, 2.57 mmol) was dissolved in 5 mL ethanol in a 50 mL quick-fit round bottom flask and stirred for 2 min, ethanolic solution of MnCl$_2$.4H$_2$O (0.25 g, 1.29 mmol) was added in drops while stirring. The flask content was then heated to reflux for 2 h during which a brown precipitate formed, which was obtained by filtration, washed with ethanol and dried under silica gel. The mass of the product was 0.20 g. The other metal complexes of NL were prepared using similar reacting masses of NL and KOH with $CoCl_2.6H_2O$ (0.31 g, 1.29 mmol), $NiCl_2.6H_2O$ (0.31 g, 1.29 mmol), and $CuCl_2.2H_2O$ (0.22 g, 1.29 mmol). The masses of the products were 0.226 g, 0.297 g, and 0.23 g, respectively. The yields and other analytical data of the compounds are found in Table 1.

RESULTS AND DISCUSSION

The products were obtained in yields of 33–48%, and stable in air. The ligand was soluble in ethanol, methanol, acetone, nitromethane, *N*-methylformamide, DMSO and DMF, and the metal complexes were soluble only in *N*-methylformamide. NL melted at 98-99°C while its metal compounds did not melt or decompose after heating up to 350°C (the maximum range of the apparatus was 360°C).

Table 1: Analytical table for NL and complexes

Compounds	Mol. Wt (g mol^{-1})	Colour	M.Pt (◦c)	Yield %	% Metal Exp (Cal)
NL	194.20	Light Brown	98-99	90	----
$[Mn(NL)_2(OH)_2]$	477.73	Brown	>360	33	11.51 (11.52)
$[Co(NL)_2(OH)_2]$	481.32	Red-Brown	>360	36	12.71 (12.24)
$[Ni(NL)_2(OH)_2]$	481.08	Light Green	>360	48	13.13 (12.20)
$[Cu(NL)_2(OH)_2]$	485.94	Green	>360	37	12.85 (13.08)

The percentage metal content by EDTA titration gave close experimental and calculated values for each metal complex (Table 1), and the stoichiometry of the complexes was determined as $M(L)_2(OH)_2$ on this basis.

^{1}H- and ^{13}C-NMR spectra

The atom labelling for the NMR spectrum of ligand NL was depicted in Scheme 1 and the spectra were shown in Figures S1 and S2. The azomethine proton absorbed as a singlet peak at 8.48 ppm,[9] of lower frequency compared to the diaryl and diphenylphosphino derivatives with 9.0 and 8.80 ppm.[3, 10] The aromatic protons (H2–H6) were observed as multiplets in the range 8.55–7.75 ppm) and the weak broad signal of O–H appeared at 4.68 δ ppm. The ethylenic protons -CH_2-CH_2- absorbed at 3.69 ppm as a sharp singlet, comparable to the frequency demonstrated in a related derivative.[10] The ^{13}C spectrum showed the peak for imine carbon (C7) at 160.47 ppm[10] and the phenyl carbon signals (C1–C6) were observed in the range 148.61–122.27 ppm while the ethylenic carbon peaks appeared at 63.56 (C8) and 60.99 (C9) ppm.[10]

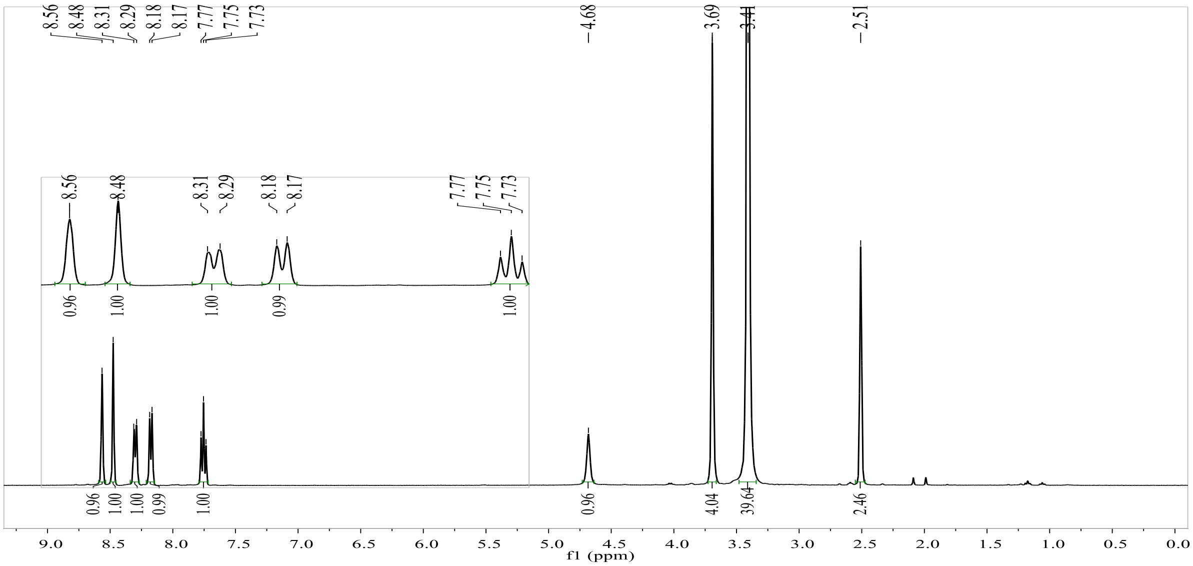


Figure S1: ^1H-NMR spectrum of NL (400 MHz, DMSO-d_6)

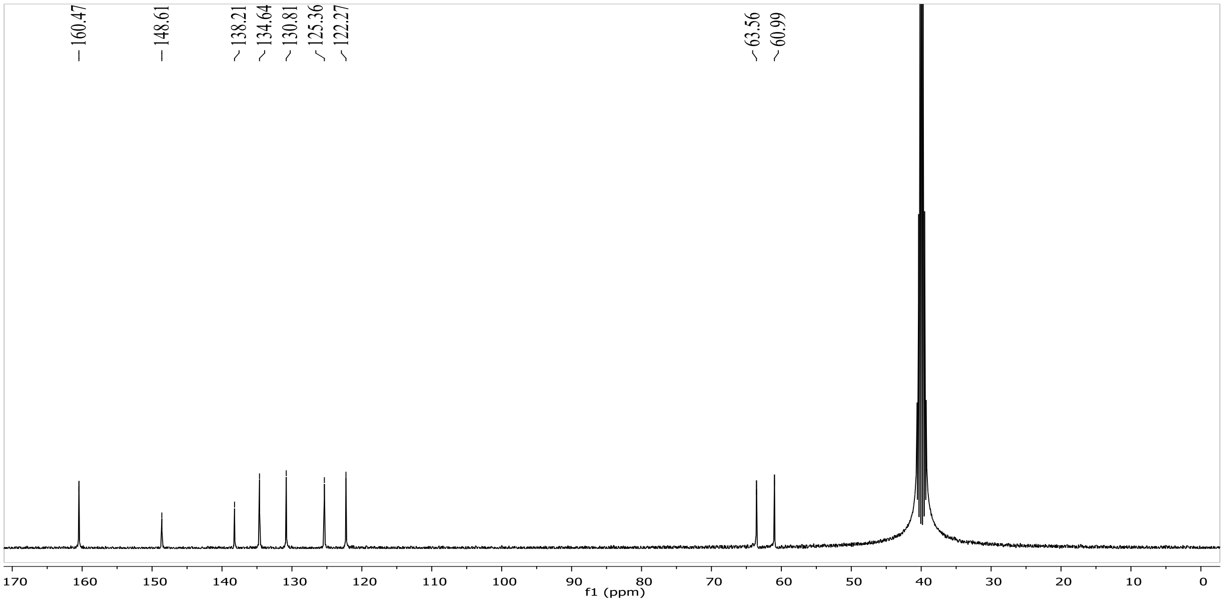


Figure S2: ^{13}C-NMR spectrum of NL (400 MHz, DMSO-d$_6$)

Infrared spectra

The infrared spectral data of the compounds were recorded in Table 2. The ligand exhibited two weak broad bands at 3381 and 3280 cm^{-1}, assigned to ethanolimine O-H and internally bonded O-H stretches. The frequency of the O-H band was lowered to 3346–3337 cm^{-1} in the metal complexes, and the new narrow bands found in the range 3641–3437 cm^{-1} were attributed to the coordinated hydroxy in the metal complexes. This suggested the coordination of the ligand through the oxygen atom and hydroxy group. The bands between 740 and 720 cm^{-1} were assigned to the O-H rocking vibration ($\delta_{(O–H)}$) in the compounds.[11] The medium bands in the region 1652–1643 cm^{-1} were allocated to C=N frequency in the compounds.[3, 12] Similarly, the band due to $\upsilon_{(C–N)}$ was not shifted, being found around 1378 cm^{-1} in the ligand and complexes.[11] The band at 1217 cm^{-1}, assigned to $\upsilon_{(C–O)}$ in the ligand spectrum,[9, 11] was shifted to 1204–1195 cm^{-1} in the complexes after coordination. These frequencies suggested the coordination of the ligand to metal ions through the ethanolimine oxygen and the non-participation of the imine nitrogen. Strong asymmetric and symmetric stretches of NO_2 appeared in the range 1529–1350 cm^{-1} in NL and metal complexes.[3] Metal to hydroxide and metal to oxygen stretches were allocated to bands found in the regions 523–499 and 407–401 cm^{-1}, and these were not observed in the ligand spectrum.

Electronic spectra

The UV/Visible spectral bands of the ligand and metal complexes were recorded in Table 2. The spectrum of NL exhibited intraligand bands at 290 and 327 nm, assigned as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, resulting from the *pi* electrons of the aromatic ring, and oxygen lone electrons in the ligand structure.[13] The bathochromic (red) shift of $n \rightarrow \pi^*$ transition in the metal complexes to the range 337-347 nm suggested the coordination of the ligand through the oxygen lone pairs. Mn(II) complex showed two bands at 562 and 478 nm and were assigned to the respective transitions $^{6}A_{1g} \rightarrow {}^{4}T_{1g}$ and $^{6}A_{1g} \rightarrow {}^{4}T_{2g}$ in the octahedral geometry. Three transitions in Co(II) complex were assigned to $^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}$ (868 nm, v_1), $^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(P)$ (635 nm, v_2) and $^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$ (562 nm, v_1) in an octahedral stereochemistry.[14] The bands at 1,068, 662 and 494 nm were ascribed to $^{3}A_{2g} \rightarrow {}^{3}T_{2g}(F)$ (v_1), $^{3}A_{2g} \rightarrow {}^{3}T_{1g}(F)$ (v_2) and $^{3}A_{2g} \rightarrow {}^{3}T_{1g}(P)$ (v_3) transitions in consistence with an octahedral Ni(II) complex.[15] A strong shoulder at 740 nm was observed close to the v_2 band, arising from weak spin-forbidden $^{3}A_{2g} \rightarrow {}^{1}E_{g}$ transition as a result of the close proximity of $^{3}T_{1g}(F)$ and $^{1}E_{g}$ terms. The 10Dq or Δ_o for this Ni(II) could be taken as 9,365 cm^{-1} (v_1).[15] A single band at 696 nm in the Cu(II) complex was ascribed to $^{2}E_{g} \rightarrow {}^{2}T_{2g}$ transition as expected in an octahedral configuration (Greenwood).[16]

Table 2: Infrared spectral data (cm^{-1}) and UV/Visible bands of NL and the metal complexes.

Compounds / cm^{-1}	$\upsilon, \delta_{(O–H)}$	$\upsilon_{(C=N)}$	$\upsilon_{(NO2)}$	$\upsilon_{(C–N/O)}$	$\upsilon_{(M–OH/O)}$
NL	3381, 3280, 738	1643	1523, 1351	1378, 1217	----
$[Mn(NL)_2(OH)_2]$	3437, 723	1652	1529, 1351	1377, 1204	515, 401
$[Co(NL)_2(OH)_2]$	3552, 3337, 724	1648	1529, 1351	1377, 1200	518, 402
$[Ni(NL)_2(OH)_2]$	3641, 3342, 721	1643	1526, 1350	1377, 1200	523, 405
$[Cu(NL)_2(OH)_2]$	3444, 3346, 737	1648	1529, 1352	1377, 1195	499, 407

	UV/Visible cm^{-1} (nm)				
Compounds	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	$d \rightarrow d$		
NL	34,490 (290)	30,580 (327)	----		
$[Mn(NL)_2(OH)_2]$	35,710 (280)	28,785 (347)	20,900 (478)	17,800 (562)	
$[Co(NL)_2(OH)_2]$	35,710 (280)	----	18,420 (543)	15,740 (635)	11,570 (868)

$[Ni(NL)_2(OH)_2]$	33,900 (295)	28,867 (346)	20,250 (494)	15,100 (662)	9,365 (1,068)
$[Cu(NL)_2(OH)_2]$	33,630 (297)	29,670 (337)	14,370 (696)		

Table 3: Zones of inhibition of the compounds (mm) against selected bacteria and fungi

Compounds	**µg/ mL**	**Bacteria**						**Fungi**			
		S. a	***B. s***	***E. c***	***P. a***	***S. t***	***K. p***	***C. a***	***A. n***	***P. n***	***R. s***
NL	1000	14	12	12	8	8	8	12	14	8	8
	500	10	10	10	8	8	8	10	10	8	8
$[Mn(NL)_2(OH)_2]$	1000	14	16	14	16	8	8	14	12	12	8
	500	12	14	12	14	8	8	10	10	10	8
$[Co(NL)_2(OH)_2]$	1000	16	14	14	14	14	14	14	14	14	12
	500	14	12	12	12	12	12	12	12	10	10
$[Ni(NL)_2(OH)_2]$	1000	16	12	14	14	12	12	14	12	10	10
	500	14	10	12	10	10	10	10	10	8	8
$[Cu(NL)_2(OH)_2]$	1000	18	16	16	16	14	14	14	14	12	14
	500	14	14	14	14	12	12	12	12	10	10
DMSO	----	8	8	8	8	8	8	8	8	8	8
Gentamicin/ Tioconazole	10	38	36	36	36	38	40	28	26	27	26

In vitro Antimicrobial Activity

The diameters of inhibition zones resulting from *in vitro* antimicrobial assay of the ligand and metal complexes were recorded in Table 3.

At sample concentrations 500 and 1000 µg/mL, NL and Mn(II) complex were only active against *Staphylococcus aureus, Escherichia coli, Bacillus, subtilis, Candida albicans and Aspergillus niger,* with inhibitory zones 10-14 mm, however, they could not inhibit *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Penicillium notatum and Rhizopus stolonifer*, as shown by 8 mm diameter. Both Co(II) and Cu(II) compounds were active against all the microbial strains with inhibition diameters between 10 and 18 mm while the Ni(II) complex was active against all the strains at 1000 µg/mL at 10-16 mm. It was observed that the metal complexes showed improved activity compared to NL. Co(II), Ni(II) and Cu(II) complexes were seen to show broad spectrum activity against both the bacterial and fungal strains, with the Cu(II) complex demonstrating the largest inhibition zone of 18 mm against *Staphylococcus aureus*.

CONCLUSION

The Schiff base ligand was prepared by reacting 3-nitrobenzaldehyde and ethanolamine under reflux conditions. The Mn(II), Co(II), Ni(II), and Cu(II) complexes were synthesized from the corresponding metal salts in an alkaline (KOH) medium. The characterisation of the ligand by ^{1}H and ^{13}C NMR spectroscopy confirmed the formation of the Schiff base compound from the *H*-C=N proton shift, as found in other related Schiff bases. The stoichiometry of the metal complexes formed was determined from the percentage metal obtained in EDTA titration. The ligand to metal coordination through the ethanolamine oxygen and hydroxide anion was inferred from the infrared vibrational frequencies observed for the ligand and complexes, hence, the ligand behaved as a monodentate ligand. The coordination of the hydroxyl group was also observed as high frequency bands between 3641 and 3437 cm^{-1}. The ligand and the complexes exhibited transitions $\pi \rightarrow \pi^*$ (290 nm) of the aromatic ring and $n \rightarrow \pi^*$ (327-347 nm) due to lone pairs of oxygen electrons whereas the visible bands in the metal complexes spectra were used to assign their octahedral geometry. Co(II), Ni(II) and Cu(II) complexes were seen to show broad spectrum activity against both the bacterial and fungal strains with inhibition zones 10-18 mm, with the Cu(II) complex demonstrating the largest inhibition zones in most cases. These compounds have shown potential activity against both bacterial and fungal strains tested when compared with gentamicin (36-40 mm) and tioconazole (26-28 mm).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Velappan, A. B., Datta, D., Ma, R., Rana, S., Ghosh, K. S., Hari, N., Franzblau, S. G., and Debnath, J., 2-

Aryl benzazole derived new class of anti-tubercular compounds: Endowed to eradicate Mycobacterium tuberculosis in replicating and non-replicating forms. *Bioorg. Chem.* 103 (2020) 104170 https://doi.org/10.1016/j.bioorg.2020.104170

2. Van Dyk, H., Jacobs, F. J. F., Kroon, R. E., Makhafola, T. J., and Brink, A., Characterisation, structural investigations and biological activity of substituted salicylidene-based compounds. *J. Mol. Struct.* 1276 (2023) 134737 https://doi.org/10.1016/j.molstruc.2022.134737

3. Zomorodian, K., Khabnadideh, S., Sakhteman, A., Bi, B. I., Mirjalili, Fatemeh, Ranjbar, M., and Zamani, L., Synthesis and antifungal activity of benzoxazole derivatives with their sar analysis by SAS-MAP. *Farm.* 68 (2020) 155-163 https://doi.org/10.31925/farmacia.2020.1.22

4. Kocsis, M., Szabados, M., Otvos, S. B., Samu, G. F., Fogarassy, Z., Pecz, B., Kukovecz, A., Konya, Z., Sipos, P., and Palinko, I., Varga, G., Selective production of imines and benzimidazoles by cooperative bismuth(III)/transition metal ion catalysis. *J. Catal.* 414 (2022) 163 https://doi.org/10.1016/j.jcat.2022.09.008

5. Liu, H., Ye, Z. -L., Cai, Z. -J., and Ji, S. -J., A multicomponent reaction of isocyanides, selenium powder and 3-aminooxetanes in pure water: green and efficient synthesis of 1,3- selenazolines, *Green Chem.* 25 (2023) 4239-4243 https://doi.org/10.1039/D3GC00708A

6. Ferlin, F., Valentini, F., Campana, F., and Vaccaro, L., Introducing the use of a recyclable solid electrolyte for waste minimization in electrosynthesis: preparation of 2- aryl-benzoxazoles under flow conditions**,** *Green Chem.* 26 (2024) 6625-6633 https://doi.org/10.1039/d4gc00930d

7. Valle-Amores, M. A., Feberero, C., Martin-Somer, A., Diaz-Tendero, S., Smith, A. D., Fraile, A., and Aleman, J., Intramolecular hydrogen bond activation for kinetic resolution of furanone derivatives by an organocatalyzed [3+2] asymmetric cycloaddition**,** *Org. Chem. Front***.** 11 (2024**)** 1028-1038 https://doi.org/10.1039/D3QO01471A

8. Huang, B., Zhang, X., Guo, Y., Tie, S., and Yang, D. A., One-Pot Three-Step Strategy Enables Robust and Efficient Synthesis of 2-Aryl Benzoxazoles from Amides, *ChemistrySelec*t 7 (2022), e202203149 https://doi.org/10.1002/slct.202203149

9. Olalekan, T. E., Akintemi, E. O., Van Brecht, B., Watkins, G. M. (2023) Synthesis, characterization and DFT studies of Schiff bases of *p*-methoxysalicylaldehyde, *Bull. Chem. Soc. Ethiop.* 37(3), 675-688, https://dx.doi.org/10.4314/bcse.v37i3.11

10. Ortega-Gaxiola, J. I., Valdés, H., Rufino-Felipe, E., Toscano, R. A., and Morales-Morales, D. Synthesis of Pd(II) complexes with P-N-OH ligands derived from 2- (diphenylphosphine)-benzaldehyde and various aminoalcohols and their catalytic evaluation on Suzuki-Miyaura couplings in aqueous media, *Inorganica Chim. Acta* 504 (2020) 119460 https://doi.org/10.1016/j.ica.2020.119460

11. Coates, J., Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry, P. 10815, John Wiley and Sons Ltd, Chichester, 2000.

12. Olalekan, T. E., Adejoro, I. A., Van Brecht, B., Watkins, and G. M., Crystal structures, spectroscopic and theoretical study of novel Schiff bases of 2-(methylthiomethyl)anilines, *Spectrochim Acta A Mol Biomol Spectrosc* 139 (2015) 385, http://dx.doi.org/10.1016/j.saa.2014.12.018

13. Oladipupo, O. E., Ibukun, D. T., and Olalekan, T. E., Synthesis and characterisation of mixed ligand dinuclear metal(II) complexes of anthranilic acid and pyridine-2-aldoxime, *Nigerian Journal of Chemical Research* 23 (2018) 39-50

14. Greenwood, N. N., Earnshaw, A., Chemistry of the Elements, 2nd ed., P. 1133, Reed Educational and Professional Publishing Ltd., Great Britan, 1998.

15. Greenwood, N. N., Earnshaw, A., Chemistry of the Elements, 2nd ed., P. 1158, Reed Educational and Professional Publishing Ltd., Great Britan, 1998.

16. Greenwood, N. N., Earnshaw, A., Chemistry of the Elements, 2nd ed., P. 1193, Reed Educational and Professional Publishing Ltd., Great Britan, 1998.